

WHAT IS CLAIMED IS:

1. A method for determining whether a compound or agent decreases the activity of a prostaglandin synthase selected from the group consisting of a microsomal prostaglandin E synthase (mPGES) and a hematopoietic prostaglandin D synthase (hPGDS) to react with a substrate to form a prostaglandin product, comprising the steps of:
  - (a) mixing the prostaglandin synthase with the substrate, a cofactor and the compound or agent;
  - 10 (b) incubating the mixture of step (a) with a stop solution comprising an agent that prevents the spontaneous conversion of the substrate into the prostaglandin product;
  - (c) incubating the mixture of step (b) with a detection reagent comprising the prostaglandin product labeled with a fluorescence label, and an antibody having the prostaglandin product as an immunogen;
  - 15 (d) illuminating the mixture of step (c) and a control mixture with plane polarized light having a wavelength at which the fluorescence label can be excited, and measuring the fluorescence polarization of the mixture of step (c) and the control mixture; and
  - 20 (e) comparing the measurements of step (d),

wherein finding the fluorescence polarization measurement of the mixture (c) is greater than the fluorescence polarization measurement of control mixture indicates the compound or agent decreases the activity of the prostaglandin synthase.

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2. The method of Claim 1, wherein the prostaglandin synthase is microsomal prostaglandin E synthase (mPGES), the substrate is prostaglandin H<sub>2</sub> (PGH<sub>2</sub>), the cofactor is glutathione (GSH), and the prostaglandin product is prostaglandin E<sub>2</sub> (PGE<sub>2</sub>).

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3. The method of Claim 2, wherein the microsomal prostaglandin E synthase is human mPGES comprising the amino acid sequence of SEQ ID NO:2.

4. The method of Claim 1, wherein the prostaglandin synthase is hematopoietic prostaglandin D synthase (hPGDS), the substrate is PGH<sub>2</sub>, the cofactor is glutathione (GSH), and the prostaglandin product is prostaglandin D<sub>2</sub> (PGD<sub>2</sub>).

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5. The method of Claim 4, wherein the hematopoietic prostaglandin D synthase is human hematopoietic prostaglandin D synthase and comprises the amino acid sequence of SEQ ID NO:4.
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6. The method of Claim 1, wherein the agent of the stop solution is  $\text{FeCl}_2$ .
7. The method of Claim 1, wherein the fluorescence label comprises fluorescein, phycoerythrin (PE), Texas red (TR), rhodamine, a free lanthanide series salt, a chelated lanthanide series salt, BODIPY, ALEXA, or CyDye.
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8. The method of Claim 7, wherein the fluorescence label is Texas red (TR).
9. The method of Claim 2, wherein the agent of the stop solution is  $\text{FeCl}_2$ .
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10. The method of Claim 9, wherein incubating step (b) has a duration of at least 30 seconds, and the incubating step (c) has a duration of at least 3 minutes.
11. The method of Claim 10, wherein the fluorescence label comprises fluorescein, phycoerythrin (PE), Texas red (TR), rhodamine, a free lanthanide series salt, a chelated lanthanide series salt, BODIPY, ALEXA, or CyDye.
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12. The method of Claim 11, wherein the fluorescence label is Texas red (TR), and the wavelength of the plane polarized excitation light is  $580 \pm 20$  nm.
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13. The method of Claim 4, wherein the agent of the stop solution is  $\text{FeCl}_2$ .
14. The method of Claim 13, wherein the fluorescence label comprises fluorescein, phycoerythrin (PE), Texas red (TR), rhodamine, a free lanthanide series salt, a chelated lanthanide series salt, BODIPY, ALEXA or CyDye.
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15. The method of Claim 14, wherein the fluorescence label is Texas red (TR), and the wavelength of the plane polarized excitation light is  $580 \pm 20$  nm.
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16. A method for determining whether a compound or agent decreases the activity of a prostaglandin synthase selected from

the group consisting of hematopoietic prostaglandin D synthase (hPGDS) and microsomal prostaglandin E synthase (mPGES) to

5 react with a substrate to form a prostaglandin product, comprising the steps of:

(a) mixing the prostaglandin synthase with the substrate, a cofactor and the compound or agent;

10 (b) incubating the mixture of step (a) with a stop solution comprising an agent that prevents spontaneous conversion of

unreacted substrate into prostaglandin product;

15 (c) incubating the mixture of step (b) with a detection reagent comprising the prostaglandin product labeled with Texas

Red, and an antibody having the prostaglandin product as an immunogen;

20 (d) illuminating the mixture of step (c) and a control mixture with plane polarized light at a wavelength of  $580 \pm 20$  nm

and measuring the fluorescence polarization of the mixture of step (c) and the control mixture at  $620 \pm 20$  nm; and

25 (e) comparing the measurements of step (d),

wherein finding the fluorescence polarization measurement of the mixture of step (c) is greater than the fluorescence

30 polarization measurement of control mixture indicates the compound or agent decreases the activity of the prostaglandin

synthase.

35 17. The method of Claim 16, wherein the prostaglandin synthase is human microsomal prostaglandin E synthase (mPGES)

comprising the amino acid sequence of SEQ ID NO:2, the substrate is prostaglandin H2 (PGH<sub>2</sub>), the cofactor is glutathione, the

prostaglandin product is prostaglandin E2 (PGE<sub>2</sub>).

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18. The method of Claim 17, wherein the agent of the stop solution is FeCl<sub>2</sub>.

19. The method of Claim 18, wherein the prostaglandin product labeled with Texas Red comprises a linker molecule to which the prostaglandin product and the Texas Red are bound.

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20. The method of Claim 19, wherein the linker molecule is selected from the group consisting of aminobutyric acid, aminocaproic acid, 7-aminoheptanoic acid, 8-aminocaprylic acid, Fmoc-aminocaproic acid, one or more  $\beta$ -alanines, an isothiocyanate group, a succinimidyl ester, a sulfonyl halide, and a carbodiimide.

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21. The method of Claim 20, wherein the linker molecule is a carbodiimide.

22. The method of Claim 16, wherein the prostaglandin synthase is human hematopoietic prostaglandin D synthase (hPGDS) comprising the amino acid sequence of SEQ ID NO:4, the substrate is PGH<sub>2</sub>, the cofactor is glutathione, and the prostaglandin product is prostaglandin D2 (PGD<sub>2</sub>).

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23. The method of Claim 22, wherein the agent of the stop solution is FeCl<sub>2</sub>.

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24. The method of Claim 23, wherein the prostaglandin product labeled with Texas Red comprises a linker molecule to which the prostaglandin product and the Texas Red are bound.

25. The method of Claim 24, wherein the linker molecule is selected from the group consisting of aminobutyric acid, aminocaproic acid, 7-aminoheptanoic acid, 8-aminocaprylic acid, Fmoc-aminocaproic acid, one or more  $\beta$ -alanines, an isothiocyanate group, a succinimidyl ester, a sulfonyl halide, and a carbodiimide.

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26. The method of Claim 25, wherein the linker molecule is a carbodiimide.

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27. A method for determining whether a compound or agent decreases the activity of human microsomal prostaglandin E synthase (mPGES) comprising the amino acid sequence of SEQ ID NO:2 to react with its prostaglandin H<sub>2</sub> (PGH<sub>2</sub>) substrate to form prostaglandin E<sub>2</sub> (PGE<sub>2</sub>), the method comprising the steps of:

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(a) mixing the mPGES with PGH<sub>2</sub>, glutathione, and the compound or agent;

(b) incubating the mixture of step (a) with a stop solution comprising FeCl<sub>2</sub>;

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(c) incubating the mixture of step (b) with a detection reagent comprising PGE<sub>2</sub> labeled with Texas Red, and an antibody having PGE<sub>2</sub> as an immunogen;

(d) illuminating the mixture of step (c) and a control mixture with plane polarized light at a wavelength of 580±20 nm and measuring the fluorescence polarization of the mixture of step (c) and the control mixture; and

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(e) comparing the measurements of step (d),

wherein finding the fluorescence polarization measurement of the mixture of step (c) is greater than the fluorescence polarization measurement of the control mixture indicates the compound or agent decreases the activity of the mPGES.

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28. A method for determining whether a compound or agent decreases the activity of human hematopoietic prostaglandin D synthase (hPGDS) comprising the amino acid sequence of SEQ ID NO:4 to react with its prostaglandin H<sub>2</sub> (PGH<sub>2</sub>) substrate to form prostaglandin D<sub>2</sub> (PGD<sub>2</sub>), comprising the steps of:

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(a) mixing hPGDS with PGH<sub>2</sub>, glutathione and the compound or agent;

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(b) incubating the mixture of step (a) with a stop solution comprising FeCl<sub>2</sub>;

(c) incubating the mixture of step (b) with a detection reagent comprising PGD<sub>2</sub> labeled with Texas Red, and an antibody having PGD<sub>2</sub> as an immunogen;

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(d) illuminating the mixture of step (c) and a control mixture with plane polarized light at a wavelength of 580±20 nm and measuring the fluorescence polarization of the mixture of step (c) and the control mixture; and

(e) comparing the measurements of step (d),

wherein finding the fluorescence polarization measurement of the mixture of step (c) is  
5 greater than the fluorescence polarization measurement of control mixture indicates the  
compound or agent decreases the activity of hPGDS.